

The emergence of antibiotic resistance in *Mycoplasma bovis* and its association with virulence and epidemiologic features of the disease in cattle

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Abstract

Mycoplasma bovis is one of the most important bacterial pathogens affecting the cattle industry worldwide, causing substantial economic losses through respiratory disease, mastitis, arthritis, and otitis. The present study aimed to characterize the antimicrobial susceptibility profile of *M. bovis* isolates collected in Israel between 2017 and 2025 and to compare these findings with those of isolates collected during 1994–2016. A total of 95 contemporary isolates were phenotypically evaluated by broth microdilution to determine the minimum inhibitory concentrations (MICs) of 12 antimicrobial agents. In parallel, *in silico* analysis of whole-genome sequencing (WGS) data from 143 historical isolates was performed to identify mutations in antimicrobial target genes. Isolates were classified as wild-type (WT) or non-wild-type (NWT) using the visual estimation approach to establish tentative epidemiological cut-off values (ECOFFs). Based on the visual estimation approach, no acquired resistance was detected to tiamulin or florfenicol. In contrast, high proportions of NWT isolates were observed for tylosin and tilmicosin (97%), oxytetracycline (92%), gamithromycin (50%), spiramycin (49%), tulathromycin (47%), enrofloxacin (46%), lincomycin (42%), and spectinomycin (29%).

Comparison with historical data revealed a substantial increase in antimicrobial resistance over time. The proportion of isolates with reduced susceptibility to macrolides increased from 84% to 97%, while fluoroquinolone resistance increased from 17.5% in the historical collection to 48% in contemporary isolates. Moreover, the proportion of isolates exhibiting resistance to at least three antimicrobial classes (macrolides, tetracyclines, and fluoroquinolones) increased from 6.3% to 43%, primarily reflecting the marked increase in fluoroquinolone resistance. The sequence type ST52 lineage, previously identified as the predominant genotype in Israeli dairy herds, was uniformly associated with genotypic resistance to macrolides and tetracyclines while retaining genotypic susceptibility to enrofloxacin.

Overall, the findings demonstrate a marked increase in antimicrobial resistance among *M. bovis* isolates in Israel over the past three decades. Although the persistence of susceptibility to tiamulin is consistent with global observations, resistance to fluoroquinolones and tetracyclines was substantially higher than that reported in Western Europe. These findings underscore the need for continuous national surveillance of antimicrobial susceptibility, evidence-based antimicrobial stewardship, and the implementation of rapid molecular diagnostic tools to

support treatment decisions. They also highlight the importance of developing alternative control strategies, including improved vaccines and novel antimicrobial agents.